Anthropogenic Heavy Metals in Organic Forest Soils –

Distribution, Microbial Risk Assessment and Hg Mobility

Staffan Åkerblom
Faculty of Natural Resources and Agricultural Sciences
Department of Environmental Assessment
Uppsala

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Abstract

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The continuous heavy metal accumulation in organic topsoils in forests will pose problems for soil microbial health for many years to come. The primary objective of this thesis was to investigate heavy metal pollution in forest soils and its effects on microbial function. Heavy metal distribution and solubility as well as field- and laboratory-derived dose-response relationships were determined. These aspects were combined to evaluate the environmental status of mor layers affected by local, regional and experimental heavy metal pollution.

Heavy metal distribution in mor layers was examined in field studies at two sites characterised by considerable local industrial pollution, one adjacent to a major road and the other beside a major steel plant. Around the road, Pb and Cu distribution indicated that these heavy metals were major pollutants, while Mo, Cr and Ni were highly elevated around the steel plant. There was considerable covariation among the metals studied. To improve the accuracy of heavy metal exposure assessment, different aspects of solubility were investigated. At the steelworks, deposition of alkaline dust had a significant influence on heavy metal solubility.

Two further studies focused on forested areas and soil material subject to low and diffuse heavy metal pollution. One simulated heavy metal concentration gradients (Cr, Zn, Mo, Pb, Ni, Cd) in mor samples, while the other was an investigation of remote areas subject to long-range aerial Hg transport.

Possible microbial risk was indicated by negative correlations between metal concentrations and soil respiration in small-scale field grids (50x50 m^2 with 10 m distance between sampling points to allow for spatial independence). Negative correlations were found in plots around the major emission sources. Laboratory-derived toxicity doses for Pb and Cu supported the indication of microbial risk around the road. At the steel plant there were negative correlations between soil respiration and Mo and Ni. Heavy metal covariation obscured the effects of individual metals in the field. Examination of microbial community structure by phospholipid fatty analysis (PLFA) revealed that metals induced synchronous uniform shifts in soil microbial community structure and soil respiration in experiments.

Measurement of Hg mobility with respect to DOC concentration in soil water leachates provided important data for modelling critical loads of Hg deposition on forest soils.

These results show the benefits of integrated evaluation of chemical and biological indicators in soil microbial risk assessment. Heavy metal pollutants still have negative effects on forest soils at major roads and steel plants, despite recent reductions of emissions.

Key words: mor layers, heavy metal, toxicity, distribution, microbial activity, solubility, effect dose, PLFA, risk assessment, Hg, organic matter

Author's address: Staffan Åkerblom, Department of Environmental Assessment,
SLU, P. O. BOX 7050, SE-750 07 Uppsala, Sweden. E-mail: staffan.akerblom@ma.slu.se

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Paper I - IV

The following thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Åkerblom, S. and Bringmark, L. Evaluating heavy metal risk for soil respiration in mor layers at a major roadway. (Manuscript)

II. Åkerblom, S. and Bringmark, L. Heavy metal distribution and relations to soil respiration in mor layer plots around the steel plant in Avesta, Sweden. (Manuscript)

III. Åkerblom, S., Bååth, E., Bringmark, L. and Bringmark, E. Experimentally induced effects of heavy metal on mor microbial function and community structure. *Biology and Fertility of Soils*. (Accepted)

IV. Åkerblom, S., Meili, M., Bringmark, L., Johansson, K., Berggren Kleja, D. and Bergkvist, B. Partitioning of Hg between solid and dissolved organic matter in mor layers. (Submitted)

Introduction

Heavy metal contamination in temporal and geographical perspectives

Heavy metal use in human historical development has dramatically altered the distribution of heavy metals in global geochemical cycles (Nriagu, 1989). These alterations can be detected in areas distant from the sources of metal emissions, for instance in polar regions (Dietz, Riget & Born, 2000; Boutron *et al.*, 2004), peat bogs (Bindler *et al.*, 1999), sediments (Gobeil *et al.*, 2001) and soils (Nowack *et al*., 2001). On a global scale, the anthropogenic emissions of Pb and Hg are exceeding natural emissions by several orders of magnitude (Weiss, Shotyk & Kempf, 1999; Hylander & Meili, 2003). However, historical patterns of heavy metal use have differed substantially to meet the needs of human cultures. Lead (Pb) was used extensively by the ancient Greeks and Romans, giving rise to the first well documented long-range transport to distant environmental archives (Weiss, Shotyk & Kempf, 1999).

Point sources often contribute to heavy anthropogenic heavy metal loads on forest soil ecosystems, *e.g*. smelters (Tyler, 1984; Haugland, Steinnes & Frontasyeva, 2002) and heavily trafficked roads (Munch, 1993). At the Gusum brass mill in Southern Sweden, the concentration gradient of Cu and Zn in the mor layer was found to be very steep, with contents falling by a factor of 500 within 6 km (Tyler, 1984). Roadside soils often show a high degree of contamination that can be attributed to motor vehicles (Harrison, Laxen & Wilson, 1981; Weckwerth, 2001). Pagotto et al. (2001) and other authors (*e.g*. Post & Beeby, 1993; Kelly, Thornton & Simpson, 1996) suggest that concentrations of the metals Pb, Cu, Zn, Cd and Ni decrease rapidly within only 10-50 m from the roadside. Urbanised areas are heavily disturbed by many different sources of anthropogenic heavy metals, rendering quantification of metal culprits difficult (Bergbäck, Johansson & Mohlander, 2001). On a local scale, metal emissions from different point sources can cause metal concentrations to reach extreme pollution levels (Nieminen, Derome & Helmisaari, 1999; Ernst *et al.*, 2004; Bååth, Diaz-Ravina & Bakken, 2005). In recent years, potential toxic metals (*e.g*. antimony and platinum) have been released to nature in higher than natural amounts (Weckwerth, 2001), with possible environmental impacts in the future.

Previously deposited heavy metals are accumulated in organic forest soils. Despite efforts to reduce emissions of heavy metals, continued inputs place an extra load on the existing pollutant levels. The residence of heavy metals (*e.g.* Pb and Hg) in many soil systems is considered to be hundreds or even thousands of years (Hissler & Probst, 2006; Klaminder *et al*., 2006).

Deposition rates of Pb and Hg in Scandinavia indicate long-range transport resulting in south-north gradients, with considerable anthropogenic inputs of Pb and Hg to remote areas. To combat long-range transport of atmospheric pollutants, an international protocol on reduced emissions of lead, cadmium and mercury was

agreed in 1998 (The Arhus Protocol on Heavy Metals, United Nations-ECE-CLRTAP). In forest ecosystems, an integrated monitoring (IM) programme is going on to provide an overview of the spatial and temporal variation in the condition of forested sites throughout Europe (more information on: http://www.icp-forests.org; 30-Jul-2006). Of particular interest in the programme is long-range transported atmospheric pollution as a stress factor in forests. Organic forest soils at the Swedish IM sites exhibit the reported national deposition pattern of Hg and Pb, with considerably higher concentrations in mor layers at southern than at northern locations (Löfgren, 2005; see also Fig. 11).

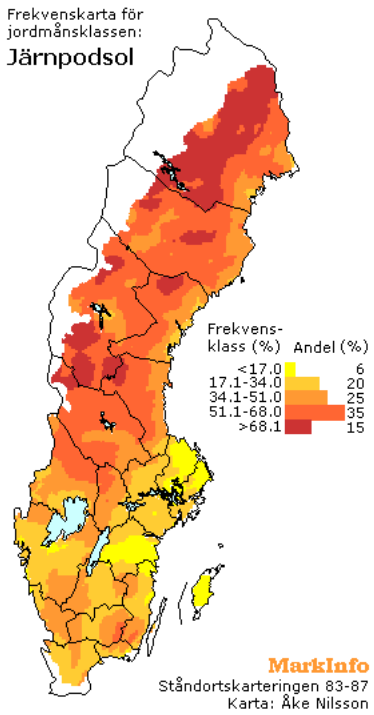

Fig. 1. Frequency of distribution of iron podsols in Sweden. (Markinfo, 2006 (www-markinfo.slu.se; 31-Jul-2006)).

Mor layers of coniferous forest soils

This thesis deals with chemical and biological processes linked to heavy metal concentrations in the organic layer of podsols (*mor* layer). A podsol is the result of hydrological and chemical processes in the forest floor (Lundström *et al.*, 2000) and is actually the dominant soil type in Sweden, covering about 70% of the total terrestrial area (Fig. 1). Organic layers are a characteristic feature of forest soils, distinguishing them from agricultural soils where cultivation destroys the layers. The mor layers are composed of a surface layer (O_i), consisting of freshly fallen leaves, needles and branches, followed by an fermentation layer (O_f) of partially decomposed litter, still recognisable as to origin and characterised by rapid mineralisation (organic matter content=40-50%) and an humification layer (O_h) consisting of well decomposed organic matter unrecognisable as to origin and characterised by a considerably slower mineralisation rate (organic matter

content=40-50%) (Hoover & Lunt, 1952). Below the O_h layer is an abrupt transition to the surface mineral soil horizon (E) containing incorporated or infiltrated organic matter (organic matter content=1-5%). The growth of coniferous trees drives the genesis of the podsol (Bockheim & Gennadiyev, 2000) since litter decomposes and leads to the formation of acidic compounds, also known as humus (Berzelius, 1839). Humus leaching from the litter and precipitates is responsible for the formation of detectable horizons in the mineral soil horizons. Soil organic matter turnover and the acidic nature and production of dissolved humus, also referred to as dissolved organic matter (DOM) or dissolved organic carbon (DOC), have an tremendous impact on element cycling (Meili, 1991; Benedetti *et al.*, 1996; Berggren, 1999; Andersson & Nilsson, 2001; Tyler & Olsson, 2002; Jansen, Nierop & Verstraten, 2004).

Effective storage of positively charged metal ions is a characteristic feature of organic soils attributed to their high concentration of negatively charged ligands in organic matter (Talibudeen, 1981). Hence, the mor layer acts as a filter and retains heavy metals supplied by atmospheric deposition. Accumulation of metals and considerable nutrient and organic matter turnover make the mor layers susceptible to metal contamination (Tyler, 1975).

Biological activity in the mor layer

Decomposition is a universal process driving geochemical cycles of nutrients and is analysed as mineralisation rate. Decomposition is the collective responsibility of soil fauna and microflora. As essential components of the environment, fungal and bacterial microbes break down dead and discarded materials, supplying a continuous source of nutrients to plants. Microorganisms are most abundant and are directly responsible for the major part of the litter breakdown, but are dependent on interactions between nutrient status, litter quality and community shifts in the soil biota (*e.g.* mites, worms and microbes) during decomposition (Wardle & Lavelle, 1997). The physical surroundings, *e.g.* moisture and temperature, are another controlling factor (Lomander, Katterer & Andren, 1998). The efficiency of biotic mineralisation is screened in activity parameters, where respiration is a general descriptor of soil biotic function. However, the quality of the carbon source decreases during mineralisation and hence the relationship between organic matter content and soil respiration is not straightforward (Berg, 2000). However, this relationship needs to be taken into account in studies of soil microbial response. The complex interactions between decomposer communities, nutrients and litter quality are also influenced by stress factors, with heavy metals as one important anthropogenic stress factor (Ramsey *et al.*, 2005).

Heavy metal stress

Heavy metals occur naturally in the environment. When heavy metals are released into the environment in higher than natural occurring concentrations, they can turn into toxic components of the environment and exert substantial stress on terrestrial and aquatic ecosystems. A loss of microbial functionality, induced by heavy metal

stress, might affect the overall cycle of nutrients and hence ecosystem productivity. In terms of environmental safety, forest soils are of concern due to their intimate links with nutrient supply and forest productivity (Tyler, 1972, 1975). However, internal microbial processes in soils have also been shown to exhibit dysfunctional changes attributable to heavy metal stress (Bååth, 1989). Furthermore, stream water chemistry is highly influenced by ecological and geological properties of the surrounding soil (Gorham, 1961), with implications for fresh water quality.

Before examination of data on ecological responses to heavy metals, an understanding of what is meant by stress is fundamental. Stress has been widely used as a descriptor of increased environmental demands on a community or body to readjust and adapt to the intensity of a stressor (Selye, 1973). Trends expected in stressed ecosystems include changes in energetics, nutrient cycling and community structure and function (Odum, 1985). Shifts in microbial function can be used to evaluate the effects of heavy metals on soil microbial ecosystems in the field (Bringmark & Bringmark, 2001b) and in the laboratory (Welp, 1999). A description of heavy metal toxicity formulated by Blanck, Wängberg & Molander (1988) uses the term *pollution-induced community tolerance* (PICT), which considers that the toxic effect does not necessarily show a functional change in *e.g.* soil respiration. Instead, the toxic effect is coupled to shifts in community composition and the evolution of tolerance (Blanck, 2002). However, the impact of microbial community structure on soil function has implications for the interpretation of microbial effects of heavy metals on soil respiration. Heavy metals are commonly considered toxic compounds in biological systems and therefore are effective stressors. However, in some cases heavy metals are also needed as trace elements, for instance copper, zinc and molybdenum. Mercury is not known to form part of any biological process.

Heavy metal solubility and mobility

Soluble fractions of heavy metals in soil are commonly considered to be more biologically relevant than the total heavy metal concentration (Ashmore *et al.*, 2000). Total soil heavy metal includes non-bioavailable forms (Almås, Mulder & Bakken, 2005). The term bioavailable fraction is often used to describe biologically relevant fractions that are responsible for heavy metal exposure of soil microbes. The bioavailable heavy metal concentration is dependent on environmental factors (Geiger *et al.*, 1996) but the dependence differs greatly among heavy metals. Solubility and availability are intimately linked. Solubility of heavy metals generally depends on the release of dissolved organic carbon (DOC) and on soil acidity (Bergkvist, Folkeson & Berggren, 1989). In aquatic toxicological science, the biotic ligand model (BLM) has been proposed to quantitatively interrelate water chemistry, physiology and toxicology to fulfil the needs of regulatory agencies (Paquin *et al.*, 2002). The same principles have been proposed for terrestrial ecosystems (Lofts *et al.*, 2004).

Determination of heavy metal exposure involves methods to make soil extracts relevant for biological effect studies (Falkengren-Grerup & Tyler, 1993; Chojnacka *et al.*, 2005) and critical examination of the ecological relevance of these fractions. Different methods are used today and involve chemical extraction (Balasoiu, Zagury & Deschenes, 2001), centrifugation (Almås, Mulder & Bakken, 2005), or lysimetric fractions (Falkengren-Grerup & Tyler, 1993). Another approach involves chemical modelling of heavy metal fractions in soil water (Lofts *et al.*, 2004). These fractions do not resemble each other with respect to their chemical composition (Giesler, Lundström & Grip, 1996) and therefore examination of their relevance is necessary (Nolan, Lombi & McLaughlin, 2003).

The recovery of soils subjected to heavy metal contamination depends on a negative mass balance, with output of heavy metals exceeding input. Detectable changes in soil concentrations levels can only be measured by long-term monitoring of soils. In organic forest soils, the principal output of heavy metals is via plant uptake and leaching, either as free heavy metal ions or complexed to DOC. Solubility and soil mass balances are closely related and depend overall on the same soil chemical principles. A close association between Hg and organic matter can be used to estimate Hg leaching in the mor layers based on DOC leaching, consistent with their strongly coupled cycling in both terrestrial and aquatic systems (Meili, 1991; Johansson & Iverfeldt, 1994; Meili *et al.*, 2003).

Monitoring of heavy metal effects on mor microbial processes

Environmental gradients are a powerful and frequently used tool in environmental research on local, regional and global spatial scale (see *e.g*. Freedman & Hutchinson, 1980; Akimoto, 2003; Steinnes, Berg & Sjøbakk, 2003). The temporal scale is also frequently monitored to record directions in the atmospheric load of heavy metals to forests (Rühling & Tyler, 2001). The environmental gradient depends on heterogeneity and variability in nature (Keddy, 1991) and is often simulated in field (Renella *et al*., 2005) or laboratory (Welp, 1999) experiments.

Environmental monitoring of heavy metal gradients often deals with ‘point sources’, *e.g*. smelter plants (Tyler, 1984; Lobersli & Steinnes, 1988) and roads (Bäckstrom *et al.*, 2003). Subsequent monitoring of biological gradients can be related to heavy metal concentrations and negative relationships between heavy metal concentrations and microbial variables are often found (Rühling & Tyler, 1979; Nordgren, Bååth & Söderström, 1983; Tyler, 1984; Post & Beeby, 1996). However, spatial problems in reconciling soil microbial effects increase on larger spatial scales (>100 m), since the influence from large-scale heterogeneities increase on the causal relationships between environmental stressors and response variables (Meentemeyer & Box, 1987). To overcome this problem, small-scale spatial relationships can be used in grid-sampling (Bringmark & Bringmark, 1998).

Relationships between soil microbiological variables and stressors in the field are commonly used to characterise the impact of pollution on the microbial community. Microbial indicators include respiration (Bringmark & Bringmark, 2001b; Niklinska, Laskowski & Maryanski, 1998), intracellular and extracellular enzyme activities (Welp & Brummer, 1997) and microbial biomass and structures (Pennanen *et al.* 1996; Muller *et al.* 2001). Reductions in microbial activities are expected to occur in the face of stress, since homeostatic processes that leave less energy available for growth and catabolic and anabolic processes are activated in organisms (Killham, 1985).

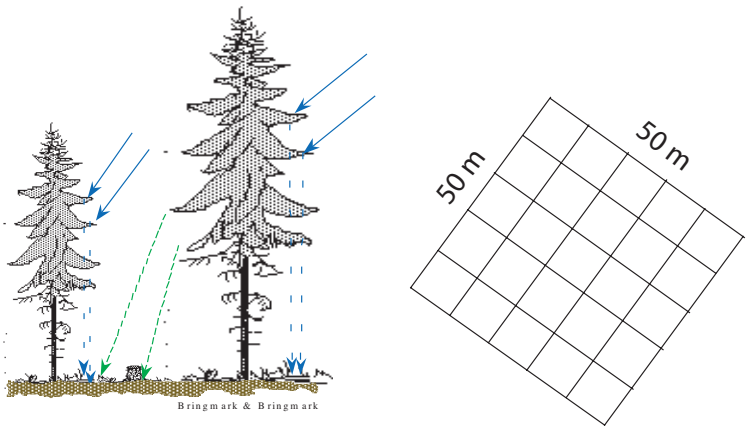

Fig. 2. Principles of sampling for spatial patterns in forest mor layers. Metals intercepted by trees were heterogeneously deposited on the forest floor directly as wet (blue lines) and dry deposition and after interception in the trees as throughfall and litterfall (green lines). Samples were taken at the nodes of regular grid systems measuring 50x50 m^2.

Homogeneous soil plots were used in the studies reported in this thesis to improve sensitivity in field spatial patterns in terms of heavy metal effects. Plots used in grid-sampling were homogeneous in the sense that they had no major breaks in soil characteristics over the plot area. The plots used measured 50x50 m^2 and samples were taken regularly every 10 m within plots to exclude autocorrelation between soil parameters, *e.g.* heavy metal concentration and soil microbial activity (Bringmark & Bringmark, 1995). Spatial variability in the soil is created by heterogeneous interception of air pollutants in the tree canopy, and subsequent deposition to the forest floor creates small-scale patterns of heavy metal contents (Fig. 2). Measuring heavy metal concentration and microbial respiration in the same sample provided the potential to look for correlations between heavy metals and microbial activity parameters within the plots. It was demonstrated by Bringmark & Bringmark (2001b) that internal negative correlations between soil respiration and heavy metal contents in soil plots were present at sites with high heavy metal loads and absent at low loads. The absence of correlations is important, as it means that the heavy metal loads are very probably causal factors. Naturally occurring heavy metals, even at high background concentrations, would not automatically result in negative relationships with respiration, a finding that can be ascribed to the evolution of

adaptation and tolerance (Smolders *et al.*, 2004). However, anthropogenically introduced heavy metals destroy naturally occurring spatial variability and, at toxic concentration levels, are likely to be seen in negative relationships to soil respiration (Bringmark & Bringmark, 1995). A lack of correlation might be expected to be more frequent in field than in laboratory studies, and at low contamination levels, as a consequence of high heterogeneity and complex interactions of the soil which link chemical, physical and biological parameters.

The toxic effect determined in experiments can be expressed in effect doses (ED), derived by linear regression between cumulative respiration and heavy metal concentrations from the experiment. Experiments performed in loess soils of low organic matter content on a wide range of heavy metals ranked the toxic actions of heavy metals based on total heavy metal concentration (Welp, 1999). Toxicity was found to decrease in the following order, with ED10 (effect dose at 10% inhibition of microbial activity; mg kg^{-1}) in dehydrogenase activity given in brackets: Hg (0.07)>Cr(VI) (2.6)>Cu (5.7)>Cd (7.7)>Ni (7.9)>Cr(III) (15)>Zn (33)>Pb (100).

Multiple stressors in polluted areas are the norm. Anthropogenic heavy metal distribution in contaminated forest soils is further often characterised by covariation among metals, which confounds interpretation of their individual environmental impacts (Suter *et al*., 2000). The toxic effects of individual heavy metals are commonly determined in laboratory studies with experimental additions of heavy metals to soil samples and subsequent monitoring of microbial activity (Welp, 1999) and microbial community structure (Frostegård, Tunlid & Bååth, 1993). Patterns in phospholipid fatty acid (PLFA) distribution can be used as indicators of microbial community composition of the soil (Sinsabaugh *et al.*, 1999). Analyses of PLFA patterns in simulated contaminated forest humus after 6 months of incubation reveal that changes are similar irrespective of heavy metal (Frostegård, Tunlid & Bååth, 1993). Changes in the microbial community structure have been found without concurrent changes in microbial activity parameters (Pennanen, 2001), but the opposite has also been reported (Ramsey *et al.*, 2005).

Long-term deposition of heavy metals induces responses in the soil microbial community that may be difficult to detect. However, the effects in forest soils may have subtle, cumulative and potentially damaging long-term implications. The challenge in soil environmental monitoring is to resolve weak signals from large ecological noise.

Objectives

The overall objective of this thesis was to evaluate aspects of the environmental impact of heavy metals in mor layers, including distribution and solubility of heavy metals and microbial risk. A further objective was to quantify leaching of mercury and control of mercury transport from mor layers.

Specific objectives were to:

- Study the distribution of heavy metals in the mor layers around two significant heavy metal point sources in Sweden, namely a busy road in Stockholm (Paper I) and a steel plant in Avesta (Paper II).

- Assess heavy metal exposure of soil biota around the point sources by means of solubility determinations using lysimeter (Papers I and II) and extraction techniques (Paper II).

- Evaluate dose-response relationships within field plots at different distances from the point sources (Papers I and II).

- Analyse heavy metal stress by addition of heavy metals to mor material in laboratory experiments, in order to calculate effect doses for soil respiration (Papers I and III) and to determine how soil respiration is coupled to changes in the microbial community structure (Paper III).

- Study the mobility of Hg in two mor layers subjected to long-range pollution and the correlation to soil organic carbon mobility (Paper IV).

General comments

In all papers in this thesis, except Paper IV, the O_f layer was evaluated in the heavy metal exposure and microbial effect assessments (in Paper IV the whole mor profile was included). The reasons for focusing on the O_f layer were mainly its high microbial activity and sensitivity to heavy metal disturbance in the soil horizon. Previous studies performed by Bringmark & Bringmark (unpublished data) indicate considerably lower respiration rates in the lower part of the mor layer (Respiration rates ratio O_h/O_f layer≈0.5). Major mineralisation processes in mor layers are disturbed by anthropogenic heavy metals (Tyler, 1975). Furthermore, the heavy metals in the O_h layer originate from old deposited material.

Pb, Cu and Zn have been identified as serious pollutants in the Stockholm region (Bergbäck, Johansson & Mohlander, 2001; Paper I). These heavy metals have been the focus of regulatory actions regarding threats to the soil environment since substantial amounts have accumulated and are still being released. Around the smelter plant in Avesta, the pattern of contamination is somewhat different to that in the Stockholm area, with Mo, Cr and Ni being major components of the industrial emissions (Papers II and III). The contamination pattern observed in areas with specific point sources (*e.g.* steelworks) is suitable for pollution studies, as certain heavy metal species are easily identified as the major pollutants. Mercury is known to be one of the most toxic compounds in nature. Despite this, knowledge of the effects on microbial processes and of how organic forest soils are affected by long-range transported atmospheric Hg is scarce. An important step in minimising the negative effects of long-range transported atmospheric Hg is to quantify Hg leaching from the mor layer for calculation of the critical load in order to keep concentrations below the level causing negative effects on soil microbial processes.

Different aspects of the heavy metal distribution were considered in the papers in this thesis. Papers I and II focused on forest soils in urban areas with considerable heavy metal contamination, while the soils and sites studied in Papers III and IV were chosen because of the low and diffuse influence of anthropogenic heavy metal deposition. The common feature in the four papers was aspects of heavy metal gradients on local scale (Papers I and II), regional scale (Paper IV), and in laboratory simulations (Paper III).

In Papers I and II, heavy metal concentrations were compared with reference sites considered not to have been influenced by anthropogenic deposition from the point sources. However, heavy metal concentrations in the mor layers of reference sites are approximate baseline levels, as regional variations in heavy metal concentrations of underlying parent material might influence heavy metal concentrations of the mor (Salminen & Tarvainen, 1997; Reimann & Garrett, 2005).

Evaluating heavy metal risk for soil respiration in mor layers at a major roadway. (Paper I)

Vehicle-derived emissions often contain, among other heavy metals, Pb, Cu and Zn (Sternbeck, Sjödin & Andreasson, 2002). The traffic sector has been a major source of anthropogenically emitted metals, with Pb as the most dominant over time (Weiss, Shotyk & Kempf, 1999; Bergbäck, Johansson & Mohlander, 2001). However, in recent years it has been suggested that Cu from brake linings might have adverse effects on the environment.

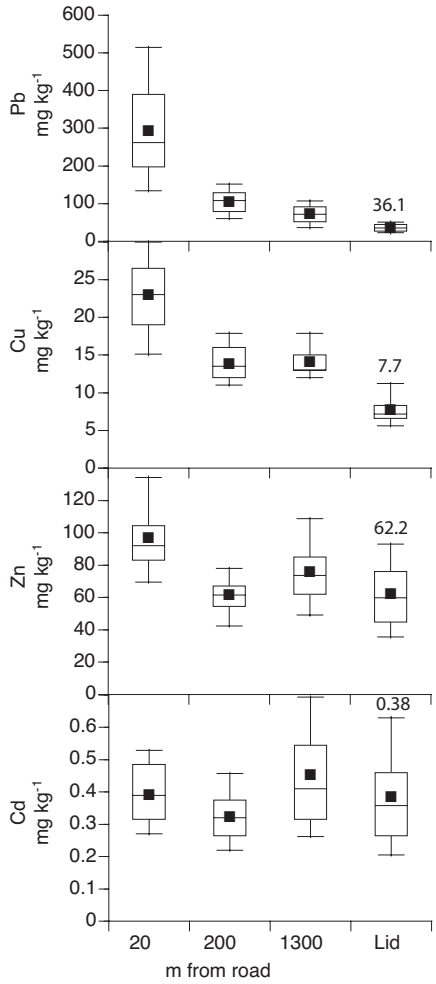

Fig. 3. Metal concentrations in O_f layers at different distances from a road (n=36) and reference site Lid (n=30; 100 km south of Stockholm). Mean values indicated with horizontal lines (and by numbers for Lid), median values with filled boxes (■), boxes show standard deviation and bars indicate 10 and 90-percentiles.

The investigation was carried out by grid sampling (see '*Monitoring of heavy metal effects on mor microbial processes*' above) of three sites at different distances from a major road in Stockholm (sites 1, 2 and 3 at 20, 200 and 1300 m from the road). The risks to microbes of Pb, Cu, Zn and Cd were studied using an environmental risk assessment approach suggested by Bates, Cullen & Raftery (2003) based on four steps, namely: (i) hazard identification, (ii) exposure assessment, (iii) dose-response assessment and (iv) risk characterisation.

Exposure in the field was assessed by determination of total heavy metal concentrations in soil samples. Assessment of the exposure was complemented with a comparison between total and dissolved metal concentrations in field sampling grids. Field site means of total heavy metal concentrations were compared with those of a reference area not affected by metal pollution (*Lid*) to determine whether heavy metal concentrations were elevated. Cause-effect small-scale spatial relationships in the field were monitored by searching for spatial correlations between total metal concentration and soil respiration in mor samples within the plots. To more accurately evaluate cause-effect relationships for individual metals, laboratory-derived effect doses were compared to field metal concentrations at different distances from the road.

Heavy metal distribution and exposure

Spatial means of Pb, Cu, Zn and Cd in the O_f layer within all sampling sites around the road showed elevated concentrations compared to a regional background value at the reference site, except for Zn within site 2 (Fig. 3). Concentrations of Pb, and to a certain degree Cu, were inversely correlated with distance from the road. Concentrations of Cu at lower depths in the soil profile are generally low, but around the basin of Lake Mälaren (near the Stockholm area) concentrations tend to be naturally elevated (Melkerud, Olsson & Rosén, 1992). This could influence Cu concentrations in the mor around the road.

In a similar study based on a smaller number of samples, Bringmark & Bringmark (2001b) reported elevated heavy metal concentrations up to 700 m away from the road and noted that Cu penetrated further into the forest than Pb. This trend was also evident in the data set of Paper I, where Pb showed a much faster decrease in concentration compared to Cu at greater distance from the road.

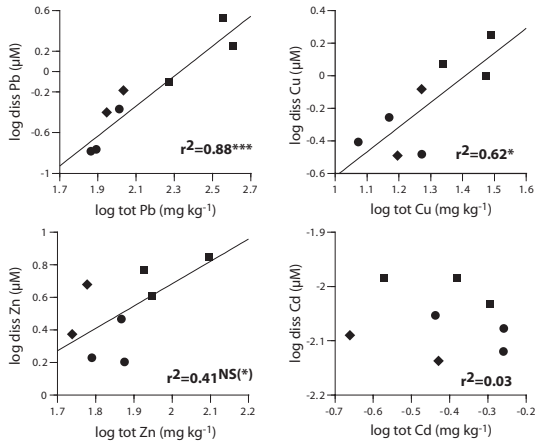

Fig. 4. Regression analysis between dissolved and total metal concentrations in bulk-samples at site 1 (■), site 2 (◆) and site 3 (●) m. Coefficient of determination (R^2) between variables and levels of significance are denoted in the figures: *p<0.05, ***p<0.001. Not significant correlations are denoted NS.

Heavy metal solubility

The dissolved fraction derived from lysimetric suction has been reported to closely resemble the heavy metal fraction available to microbiota in soil (Bergström &

Stenström, 1998). The strong linearity between total and dissolved heavy metal concentrations of Pb, Cu and Zn in bulk samples (Fig. 4) gives reason to believe that this correlation also existed in the smaller mor samples used for respiration measurements. For this reason, total heavy metal content was used as a substitute for the more bioavailable dissolved heavy metal concentrations in dose-response relationships in field sites. For Cd, the variation in both total and dissolved heavy metal concentrations was low, even between sites situated close to and distant from the road. The absence of linearity was probably the result of absence of variation in total and dissolved heavy metal concentrations.

Microbial risk assessment

The microbial risk assessment in Paper I was not solely based on individual results of *e.g*. exposure and field dose-response relationships. Instead, the environmental risk was characterised by a sequential elimination of heavy metals considered to be of low potential risk.

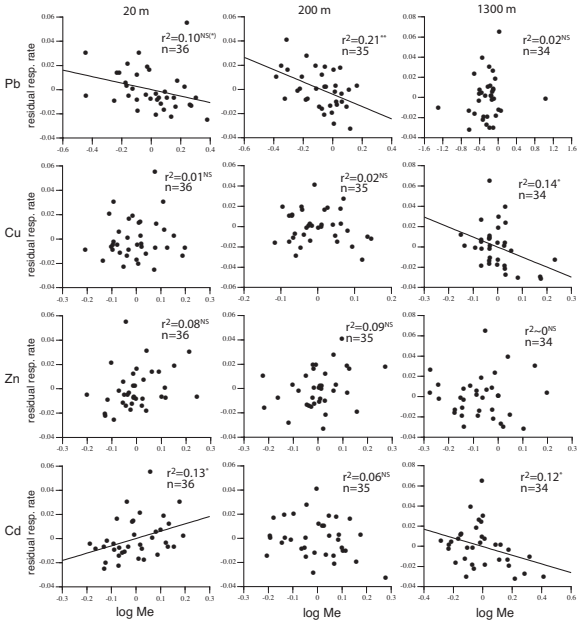

Fig. 5. Spatial relationships between metal levels (logarithmically-transformed values) (mg kg^{-1}) and respiration rate at different distances from road. Due to high minerogenic content in mor-samples, all variables were represented by residuals of the relationship with organic matter in soil samples. Coefficients of determination (R^2) between variables and levels of significance are denoted in the figures: *p<0.05, **p<0.01, *** p<0.001. Not significant correlations are denoted NS.

The high degree of contamination by Pb suggests that it could pose a risk to soil microbial function (Tyler, 1975). Pb affected microbial activity and was a dominant heavy metal pollutant in the top organic layers of forest soils in close proximity to the road. Microbial effects for Cu were weaker than for Pb, but

seemed to extend a longer distance from the road (Figs. 5 and 6). The effect of Zn on microbial processes was not confirmed by our results. However, effect doses indicated possible detrimental effects. The Cd concentrations in the mor around the road gave no indication of any negative effects on microbial processes in the top organic soil layer.

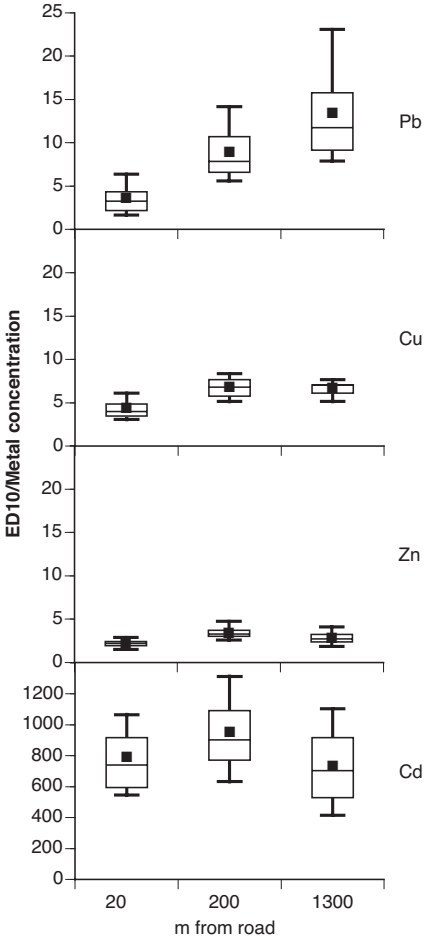

Fig. 6. ED10/Me in O_f layers at different distances from a road (n=36). Mean values indicated with horizontal lines, median values with filled boxes (■), boxes show standard deviation and bars indicate 10 and 90-percentiles. (Note the different scale on the Y-axes for Cd)

Heavy metal distribution and relations to soil respiration in mor layer plots around the steel plant in Avesta, Sweden. (Paper II)

Emissions of heavy metals in Paper II were restricted to a well-defined point source, namely a steel plant in Avesta, Sweden, compared to the heavy but more diffuse emissions from traffic in Paper I. Two field sites ('Factory' at 600 m and 'Intermediate' at 3 km from the main emission source) in the surroundings of the steel plant were sampled by grid sampling methodology and compared with a reference site further away (27 km).

The aim of Paper II was to evaluate the degree to which forest sites were affected by heavy metal pollution from the steel plant and whether soil respiration was affected by heavy metal pollution. Furthermore, exposure levels were compared with respect to total and available heavy metal fractions in the soil and we also examined whether the exposure was affected by chemical properties within the sampling site. The Factory site was severely disturbed by high loads of alkaline dust increasing pH to unnaturally high levels (pH=7.3).

Table 1. *Total (n=36 except Reference site n=12) (µg g^{-1}), exchangeable (µg g^{-1}) and lysimetric (µg L^{-1}) (bulk samples; n=4) metal concentrations of O_f samples (mean values and standard deviation (SD)). Elevation ratios (ER) describe the elevation of metal concentrations within soil plots above metal concentrations of Reference site.*

		Factory			Intermediate			Reference	
		Mean	SD	**ER**	Mean	SD	**ER**	Mean	SD
Fe	Total	10553	492	**8.8**	2804	117	**2.3**	1197	76
	Exchangeable	0.106	0.002	**2.2**	0.112	0.001	**3.0**	0.038	0.001
	Lysimeter	235	193	**0.5**	1000	405	**2.1**	468	122
Mo	Total	277	163	**113**	31.0	14	**13**	2.4	0.91
	Exchangeable	0.443	0.005	**1585**	0.002	0.001	**7.6**	$0.24\ e^{-4}$	$0.05\ e^{-4}$
	Lysimeter	844	473	**1094**	7.9	2.7	**10**	0.77	0.17
Cr	Total	733	279	**98**	83.3	30	**11**	7.4	2.0
	Exchangeable	0.014	$3.6\ e^{-3}$	**5.1**	0.003	0.001	**1.2**	0.003	0.001
	Lysimeter	19.2	2.3	**5.5**	17.6	3.5	**5.0**	3.5	1.1
Ni	Total	560	157	**45**	129	40	**10**	12.4	6.2
	Exchangeable	0.022	$4.0\ e^{-3}$	**1.0**	0.226	0.059	**10**	0.022	$3.7\ e^{-3}$
	Lysimeter	50.8	16.3	**8.2**	32.5	5.9	**5.2**	6.2	2.7
V	Total	26	8	**5.6**	7.5	2.2	**1.6**	4.6	1.3
	Exchangeable	0.002	$5.2\ e^{-4}$	**1.5**	0.002	0.001	**1.7**	0.001	$2.4\ e^{-4}$
	Lysimeter	1.8	0.5	**0.5**	5.7	1.1	**1.4**	4.1	1.1
Pb	Total	79	28	**2.3**	48	13	**1.4**	34	12
	Exchangeable	0.003	0.001	**0.05**	0.118	0.1	**2.1**	0.056	0.028
	Lysimeter	0.68	0.36	**0.33**	2.2	0.9	**1.09**	2.0	0.9
Cu	Total	47.3	9.0	**6.1**	14.5	2.5	**1.9**	7.8	1.4
	Exchangeable	0.012	$1.4\ e^{-3}$	**2.1**	0.007	0.001	**1.1**	0.006	0.001
	Lysimeter	9.9	4.5	**0.74**	14	3	**1.1**	13	3
Zn	Total	210	50	**3.3**	61	10	**0.90**	64	11
	Exchangeable	0.022	$1.5\ e^{-3}$	**0.03**	0.762	0.145	**1.0**	0.750	0.053
	Lysimeter	7.8	2.1	**0.09**	74	16	**0.86**	85	20
Cd	Total	2.1	0.5	**5.3**	0.4	0.2	**1.0**	0.4	0.1
	Exchangeable	0.002	0.001	**0.23**	0.005	0.001	**0.80**	0.007	0.001
	Lysimeter	1.8	1.0	**3.5**	0.43	0.10	**0.85**	0.50	0.11

Compared to Paper I, the microbial risk assessment was restricted to field dose-response relationships and determinations of heavy metal elevations. Small-scale relationships between soil respiration and heavy metal concentrations were determined using partial least square (PLS) analysis, which is suitable as it benefits from multiple colinearities in the explanatory variables (heavy metals).

Heavy metal distribution and exposure

Highly elevated total concentrations of Mo, Cr and Ni were found in the immediate vicinity of the steel plant, compared with the reference site (Table 1). At the Intermediate site, the elevations of total concentrations of Mo, Cr and Ni were more moderate, but were still about tenfold the background concentrations, indicating severe contamination and high potential risk. In contrast to total concentrations, pore water Cr and Ni concentrations were only moderately elevated at the Factory site. Pore water Mo concentration at the Factory site was almost tenfold greater than the already highly elevated total concentration. Thus, pore water concentrations of Mo strengthened the diagnosis of severe environmental risk at this site. However, at the more distant Intermediate site, Mo pore water concentration was less elevated than total Mo concentration.

The strong covariation among the heavy metals within grids confirmed the assumption that the observed elevated concentrations most probably originate from a common source of contamination (Spijker, Vriend & van Gaans, 2005) or similar mode of forest canopy interception and transport to the forest floor.

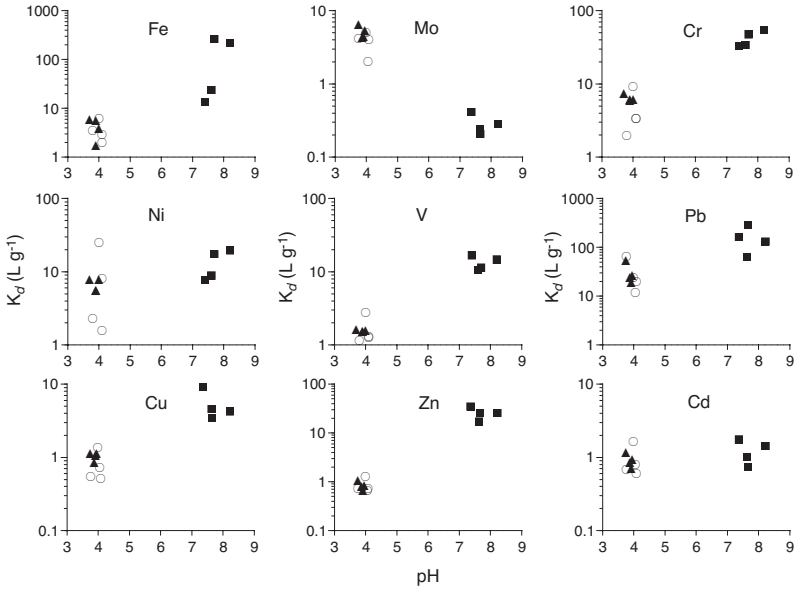

Fig. 7. Heavy metal partitioning of total to pore water heavy metal concentrations (K_d, L g^{-1}), as functions of pH. Fe, Mo, Cr, Ni, V, Pb, Cu, Zn and Cd in bulk samples from three sites at Avesta steelworks (Factory site (■), Intermediate site (▲) and Reference site (○)) (Paper II).

Heavy metal solubility

The solubility of pore water heavy metal concentration in bulk samples was expressed as partitioning coefficient relating total heavy metal concentration to pore water heavy metal concentration (K_d (L g^{-1})) and values obtained are

summarised in figure 7. At the alkaline Factory site Fe, Cr, V, Pb, Cu and Zn were less soluble but Mo showed considerably higher solubility, consistent with data on Mo solubility characteristics in neutral and alkaline conditions (Gustafsson, 2003), compared to plots further from the steel plant. The solubility patterns resulted in the heavy metal exposure described above.

An interesting finding in the solubility determinations was that K_d for Cd did not differ between sites, despite the large difference in pH at the Factory site. This pattern conflicts with previous findings on Cd solubility and its relation to soil acidity (Bergkvist, Folkeson & Berggren, 1989). Previous studies performed by Berthelsen, Olsen & Steinnes (1995) showed considerable accumulation of heavy metals in the ectomycorrhizal biomass of top forest soil. Therefore, we reasoned that ectomycorrhizae might contribute more to Cd storage at the Intermediate and Reference sites than at the highly disturbed Factory site.

Microbial risk assessment

The consequence of the very high concentrations of Mo, Cr and Ni, especially at the Factory site, is a high risk of deleterious effects (Tyler, 1975). Considering the solubility of Mo at this site, the risk is even higher.

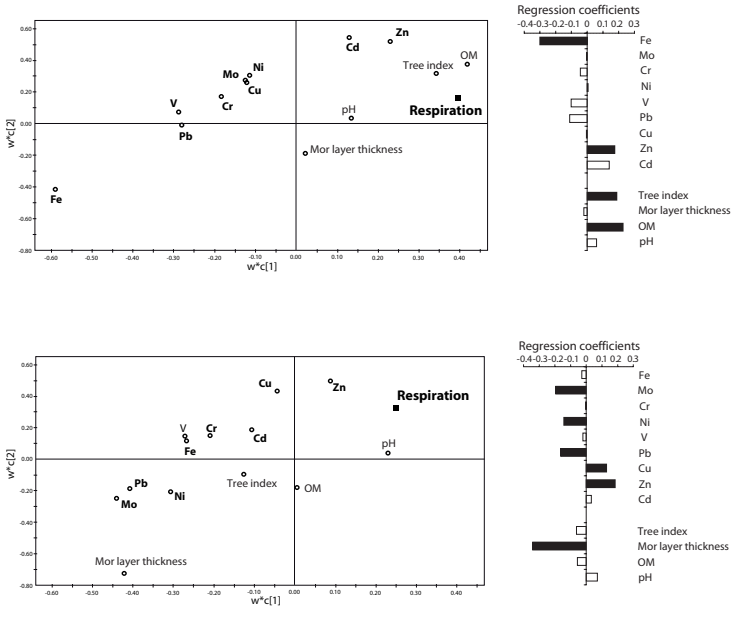

Fig. 8. PLS analysis at the Factory (top) and Intermediate (bottom) sites of respiration response to environmental variables (heavy metal concentration, organic matter content (OM), pH and tree index), with PLS regression coefficients in histograms to the right. Significant (VIP>1) coefficients are indicated by filled columns.

At the Factory site, negative correlations of heavy metals to respiration were not verified, except in the case of Fe (Fig. 8). At the Intermediate site, the negative correlations between heavy metal concentrations and respiration were more

pronounced. High concentrations of Mo and Ni and negative relationships with respiration are reasons for concluding that toxic effects on microbial function are probable, with consequences on mineralisation and nutrient cycling. Pb had a negative relationship to respiration but was not elevated in either total or soluble Pb fractions, and was therefore not considered to pose a risk to soil microbial function.

Experimentally induced heavy metal effects on mor microbial function and community structure. (Paper III)

Of the three papers in this thesis with particular interest in microbial effects (Papers I-III), Paper III focused mainly on evaluation of the microbial response. To explore how individual heavy metal species relate to heavy metal concentrations in the field, simulated heavy metal concentration gradients were created in mor samples collected in areas with low baseline concentrations. The microbial activity was monitored as soil respiration for 64 days. At the end of the period, the microbial community structure was analysed by PLFA composition patterns.

To quantify the heavy metal effect, effect doses were calculated from the cumulative respiration in the experiment.

Heavy metal exposure

The heavy metals were added to the mor samples in appropriate amounts to attain linearly increasing concentrations among the dose levels (Table 2, also including Cu from Paper I). Heavy metal increments in treatments differed for different heavy metals, and were based on previous experience and reported heavy metal toxicity data. As regards pollution levels found around the road (Paper I) and the steel plant in Avesta (Paper II), heavy metal concentrations and increments in low and medium dose levels were environmentally relevant for all heavy metals except for Cd. In the case of Cd, previous experiments have found minor effects of Cd on soil microbial function even at high concentrations (Niklinska, Laskowski & Maryanski, 1998).

Table 2. *Metal concentrations (mg kg^{-1}) and elevation ratio in dose levels in experiments relative to controls (ER) of Cr, Cu, Zn, Pb, Mo, Ni, and Cd in controls and treated soils.*

	Cr		Cu		Zn		Pb		Mo		Ni		Cd	
	mg kg^{-1}	**MI**	mg kg^{-1}	**MI**	mg kg^{-1}	**MI**	mg kg^{-1}	**MI**	mg kg^{-1}	**MI**	mg kg^{-1}	**MI**	mg kg^{-1}	**MI**
Control	6.0	**1**	7.5	**1**	82.6	**1**	43.9	**1**	2.5	**1**	6.0	**1**	0.36	**1**
Low	20.9	**3.5**	11.2	**1.5**	142	**1.7**	86	**2**	7.1	**3**	17.7	**3**	9.8	**27**
Middle	155	**26**	44.5	**5.9**	579	**7**	261	**6**	46.0	**19**	123	**20**	94.4	**260**
High	1553	**259**	378	**50**	4283	**52**	2029	**46**	460	**188**	1230	**205**	940	**2600**

Microbial risk assessment

Paper III explained two interesting features relevant in the microbial risk assessment procedure. Firstly, for all heavy metals there was a strong uniformity

in the microbial community structure response to heavy metal stress (Fig. 9). However, in addition to the strong uniform structural shift along the first principal component there was also a noticeable shift along the second principal component, with Cr and Cd most strongly separated. Secondly, soil respiration and the microbial community structure responded synchronously to the heavy metal stress, irrespective of the heavy metal tested (Fig. 10).

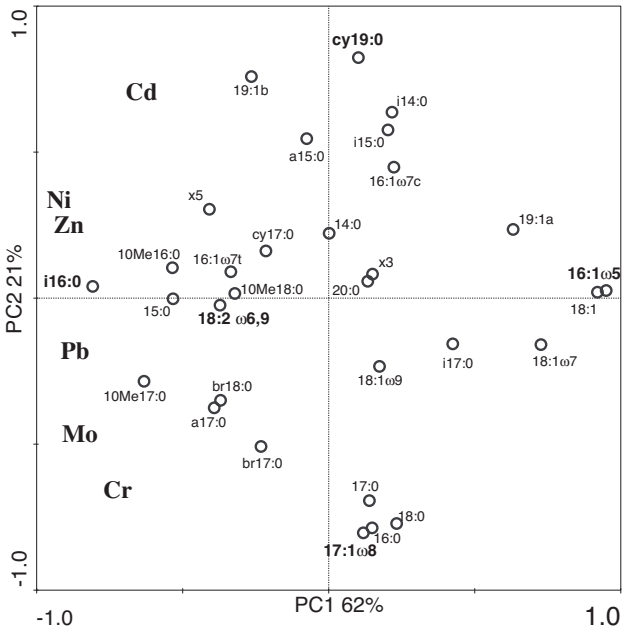

Fig.9. PCA based on loadings for the individual PLFAs from PC1 of separate RDA of each heavy metal (PC1 of RDAs accounted for 42%, 23%, 7%, 15%, 12% and 11% of the variation in the data set for Cr, Zn, Pb, Mo, Ni and Cd, respectively).

The shape of the dose-response curves (Fig. 10) differed among heavy metals. For example, Cr and Zn presented a classical sigmoid dose-response curve with inhibition of respiration and induced shifts in the microbial community structure linked to heavy metal stress, even at low heavy metal increments. Microbial activity for Pb and Mo fluctuated at low and medium doses, and the negative effect on microbial activity was only observed at high doses. In the case of Ni and Cd, treatments with low and high doses resulted in shifts in the microbial community structure in different directions relative to the control, suggesting a lack of a unidirectional concentration-dependent response to Ni and Cd stress. The coherent shift in microbial respiration further suggests that microbial community and microbial activity have significant implications in the evaluation of toxicity experiments.

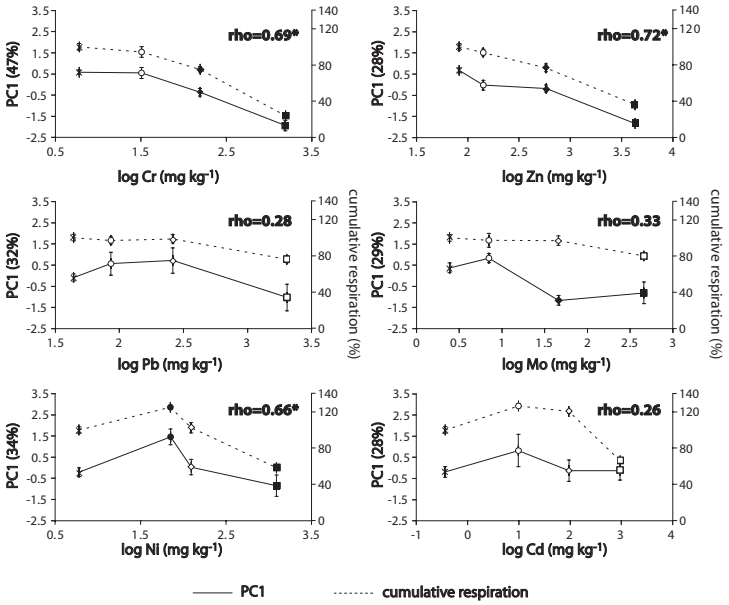

Fig. 9. Microbial community structure (sample scores of the first *principal component* (PC) of the PLFA pattern) and cumulative respiration as a function of heavy metal concentrations in experiments (logarithmic heavy metal concentrations for Cr, Zn, Pb, Mo, Ni, and Cd). Percentages on left axes indicate variation explained by PC1. Spearman rank correlation coefficients (rho) between microbial community structure and cumulative respiration in all samples were calculated. Significant correlations are denoted *. Filled symbols differ significantly from controls (Anova, followed by Dunett's test for comparison with controls). Error bars in figures denote standard error (controls (x) n=12; low (○) n=4; medium (◇) n=4 and high dose level (□) n=4). α=0.05

Effect doses are commonly described in terms of heavy metal concentrations. Ranking of heavy metal toxicity based on total doses ignores the effect of microbial community structures naturally occurring at background heavy metal concentration levels in the soil. Paper III therefore described an alternative approach of expressing effect doses as increments relative to initial heavy metal concentrations in controls. Ranking of toxicity by taking background concentrations into account provides a better description of the risks in comparisons of heavy metals as described in Paper I. As different soil systems can have different natural background concentrations, the resident microbial community has different sensitivities and may thus respond differently to heavy metal loads. In environmental extrapolation of laboratory toxicity data this effect needs to be taken into account, as the associated variability has important consequences if soils are under serious environmental risk.

Partitioning of Hg between solution and solid phase in organic forest top soils. (Paper IV)

The aim of Paper IV was to study the mobility of Hg in mor layers and to relate it to mobility of DOC, since it has been shown that there is a strong link between Hg and organic matter in terrestrial Hg cycling (Meili, 1991; Johansson & Iverfeldt, 1994). The field study was conducted at a forest site in southern Sweden with high Hg deposition (Aneboda) and a site in northern Sweden with low deposition (Gammtratten).

Compared with the previous field studies reported in Papers I and II, the anthropogenic contribution of Hg in the mor layers studied in Paper IV was more diffuse and originated from long-range atmospheric pollution. Total deposition compared to open field wet deposition clearly showed that the forest canopy provides an important additional flux of Hg to forest soil ecosystems. In fact, total annual Hg deposition at Aneboda (40 µg m^{-2}) and at Gammtratten (12 µg m^{-2}) (Löfgren, 2005) was four times the flux of open field wet deposition in adjacent areas (10 and 4 µg m^{-2} $year^{-1}$ in southern and northern Sweden, respectively), which was an higher additional input than recorded in other studies (Grigal *et al.*, 2000; St. Louis *et al.*, 2001). The additional input is most likely from dry deposited Hg on trees and then washed off plant surfaces, elevating concentrations of Hg in throughfall over those in precipitation (Iverfeldt, 1991). Furthermore, the

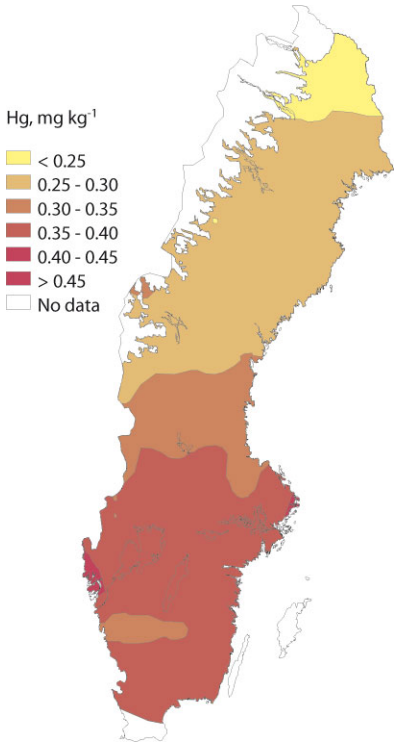

Fig. 11. Hg concentrations in mor layers of Sweden (Naturvårdsverket, 2002).

Table 3. *Solid phase characteristics in the mor layer of two Swedish forest sites (Aneboda and Gammtratten). Range, mean, median, and coefficient of variation (CV%) for thickness of the mor layer, soil organic carbon concentrations (SOC), Hg_s concentrations (Hg_s), and Hg_s /SOC-ratios based on composite mor layer samples (n=6) and vertically divided mor layer samples (O_f-layer and O_h-layer, n=6, respectively).*

		Mor layer thickness (cm)			SOC (%)			Hg_s (µg g^{-1})			Hg_s/SOC (ppb)		
		Min/max	median	Mean CV(%)	Min/max	median	Mean CV(%)	Min/max	median	Mean CV(%)	Min/max	median	Mean CV(%)
Aneboda	Mor layer	4/12	5	6.2 (47)	38.8/48.8	46.6	44.8 (9)	0.26/0.42	0.35	0.34 (17)	0.54/0.93	0.75	0.76 (18)
	O_f-layer				47.7/49.9	48.5	48.6 (2)	0.22/0.42	0.31	0.31 (23)	0.47/0.84	0.63	0.63 (21)
	O_h-layer				25.6/46.1	42.1	38.2 (22)	0.22/0.60	0.39	0.41 (35)	0.86/1.45	0.48	1.05 (22)
Gammtratten	Mor layer	6/12	8	8.5 (24)	42.6/47.5	44.7	44.8 (5)	0.26/0.38	0.30	0.31 (16)	0.56/0.89	0.67	0.70 (17)
	O_f-layer				47.5/49.9	49.0	48.9 (2)	0.18/0.33	0.24	0.25 (20)	0.37/0.69	0.48	0.50 (21)
	O_h-layer				27.5/49.3	38.8	38.7 (20)	0.20/0.44	0.26	0.30 (34)	0.40/1.51	0.67	0.83 (49)

amount of Hg deposited by litterfall is significantly greater than that deposited by throughfall (Munthe, Hultberg & Iverfeldt, 1995). Considering the known deleterious effects of Hg on forest soil microbial function (Bringmark & Bringmark, 2001a), Hg loads on Nordic boreal forest soils are significant and serious.

Hg distribution

Previous mapping of Hg deposition showed a clear south-north gradient with considerably elevated deposition of Hg in southern Sweden (Rühling & Tyler, 2001), leading to higher Hg concentration levels in the mor layers in southern Sweden compared to the north (Alriksson, 2001) (Fig. 11). The large-scale gradient observed in soil solids was also seen in the soil solution. However, considerable seasonal variation was found in Hg concentrations of percolating soil water. Paper IV further reported a vertical Hg concentration gradient, with considerably higher concentration in the O_h layer than in the O_f layer, which has implications for the Hg content of the DOC leaving the mor layer (Table 3).

Hg mobility

Hg mobilisation from the mor layers of the forest soils investigated was closely associated with DOC mobilisation, with a strong positive correlation between Hg_D and DOC at both Aneboda and Gammtratten. At Aneboda, the correlation between Hg_D and DOC differed in tension and zero-tension lysimeters, with considerably higher Hg in the former at a given DOC value (Fig.12).

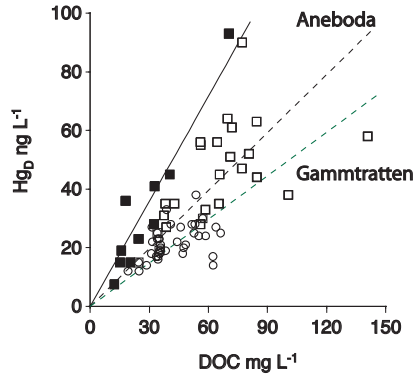

Fig. 12. Linear correlation between DOC and Hg_D in tension lysimeters (filled symbols) and zero-tension lysimeters (open symbols) at Aneboda (□) and Gammtratten (○).

The most striking result of Paper IV was that the Hg content of organic matter in solution relative to that in solids did not differ between forest sites, despite differences in Hg deposition. Using the consistency in ratios of Hg in dissolved and solid organic matter, critical load of Hg in mor layers can be calculated when data on DOC concentration and water flow are available (Schütze *et al.*, 2004).

Modelling of Hg leaching from the mor layer is important for future calculation of Hg critical load in order to establish acceptable Hg concentrations without negative effects on soil microbial processes.

Temporal trends in Hg concentrations in mor layers respond only slowly to changes in deposition, as reported by Johansson, Bergbäck & Tyler (2001). The response to decreasing Hg deposition as monitored in soil Hg concentrations is not immediate. Calculation of mass balances of the soil system is a sensitive tool for predicting the outcome of changes in deposition patterns, since negative mass balances include processes regulating carbon and Hg turnover.

Methods for soil microbial risk assessment

In the studies reported in this thesis, a risk assessment of forest soils was carried out to quantify the critical levels of heavy metals for soil microbial activities. This process generally follows consecutive steps involving exposure and effect assessment, followed by risk characterisation (Paper I). The quantification of microbial risk followed a model schematically described in figure 13. Each step included crucial decisions and combination of results to rule out heavy metals considered not to pose a risk to soil microbial systems. Other approaches for assessing the risk of heavy metals in soils have also been proposed, *e.g.* the biotic ligand model (BLM) (Paquin *et al.*, 2002).

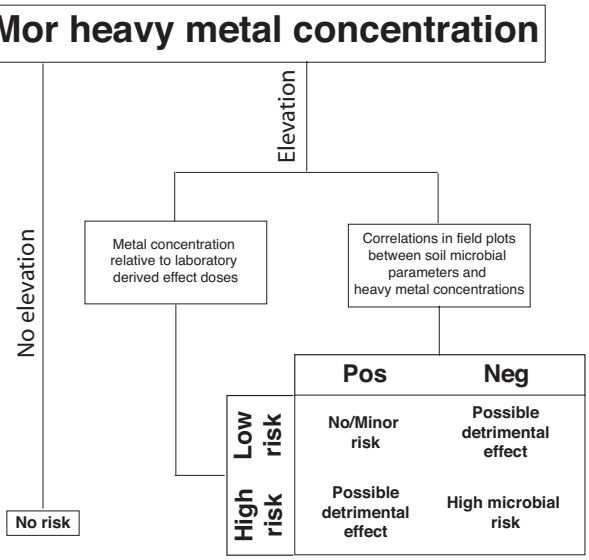

Fig. 13. A conceptual model for establishing microbial risk in organic forest soils contaminated with heavy metals.

Heavy metal contamination is commonly quantified by comparisons of total heavy metal concentration with a reference or control concentration. Characterisation of heavy metal concentration involves some important features to describe the deviation from naturally occurring concentrations (Reimann & Garrett, 2005). Therefore, the first crucial decision is to determine whether heavy metal concentrations are elevated above the reference value. The anthropogenic influence of heavy metals from local sources on reference sites used in this thesis was not investigated. Instead, the reference sites were presumed to be unaffected due to their location in the landscape. An alternative approach would be to use regional data from large-scale inventories and estimations of naturally occurring heavy metals, as proposed by the Swedish Environmental Protection Agency (Naturvårdsverket, 1999).

Variation in elevation of heavy metal concentrations among field plots studied (Paper II) led to soluble heavy metal fractions presenting a different picture of the heavy metal distribution than total heavy metal concentrations. These patterns most probably affect the toxicity of the heavy metals and hence need to be taken into account in the evaluation of soil status in figure 13. Although a proportion of the total quantity of heavy metals is potentially available to soil biota, the bioavailable (soluble) fraction is the best predictor of acute toxicity to organisms (Reimann & Garrett, 2005). We propose the use of pore water and exchangeable heavy metal fractions as supplementary information in the risk assessment, since available data on comparable background concentrations in these fractions are scarce. Extractable heavy metal concentrations show seasonal variations that are not easily interpreted (Niskavaara *et al*., 1997). Furthermore, computer simulated speciation of soil heavy metals is used more frequently in soil risk assessment today, *e.g*. BLM (Paquin *et al*., 2002) and critical limits (Lofts *et al*., 2004).

The implementation of laboratory-derived effect doses (ED) in figure 13 intimately links field concentrations and toxicity. Estimation of the magnitude of the ratio of ED to field heavy metal concentration (ED10/Me) possibly constitutes the greatest challenge of the microbial risk variables included. There is seldom financial support or time to perform artificial heavy metal toxicity testing relevant to the studied field situation. Instead, extrapolations are made from scientifically based toxicity data from previously published studies, which do not necessarily apply to the field situation under study. The ED level is always connected with uncertainty, which is commonly handled by the introduction of safety factors (Chapman, Fairbrother & Brown, 1998). The novel approach described in this thesis of comparing the relative levels of ED with field concentrations, rather than absolute values, excludes the need for safety factors.

Correlations between microbial parameters and heavy metal concentrations in field plots improve the sensitivity in field risk assessment. Respiration is often correlated to other soil microbial processes (such as enzyme activity and nitrogen mineralisation) (see *e.g*. Simona, Angela & Amalia, 2004; Böhme, Langer & Böhme, 2005). Instead of using a set of soil microbial activity parameters as proposed by Torstensson, Pell & Stenberg (1998), soil respiration was chosen as a sufficient predictor in the present studies. The heavy metal stress expressed at field

sites was apparent in negative correlations between heavy metal concentrations and soil respiration. Efforts were made to process a large number of samples collected by grid sampling. The relevance of using soil respiration as a sole predictor of soil microbial quality is primarily its reproducibility and ecological relevance, as it reflects a major biogeochemical process in the soil.

Numerous co-varying heavy metals are the norm in field environmental monitoring, posing problems in distinguishing relevant field dose-response relationships. The immediate response to additions of heavy metals, as seen in experiments (Paper III), is easily detected compared to effects in a field plot subjected to continuous loads of atmospheric emissions. The continuous long-term presence of multiple stressors has a subtle and cumulative effect on both a temporal and spatial scale. Furthermore, regional (or global) pollution patterns are masked by other geographical patterns on a landscape scale. Irrelevant stress factors, therefore, confuse the dose-response studied within and between field plots.

Additional microbial response variables in grid sampling methodology should include microbial community structure data. The possibility of using microbial community indices in evaluation of heavy metal stressed field soils is promising. Microbial effects in grids exposed to the highest heavy metal loads did not express any negative spatial dose-response correlations in Papers I and II. Data on the spatial structure of microbial community structures could further strengthen the microbial risk assessment. However, interpretation of PLFA patterns in field plots for single heavy metal effects would be confounded by the uniformity of the microbial community response to heavy metals (Paper III).

The integrated results from field and laboratory can result in several possible outcomes. There are no definite borders in the positive and negative correlation in field dose-response relationships. Furthermore, the magnitude of the risk expressed in ED10/Me is not comparable between different laboratory-derived ED. However, a negative field correlation and a high risk ED10/Me (low ratio) do add up to a high possibility of risk to soil microbial function (*e.g.* for Cu at plots distant from the road in Paper I). On the other hand, a positive field correlation and low risk ED10/Me (high ratio) most likely add up to a conclusion of low possibility of risk (*e.g.* for Pb at plots distant from the road in Paper I). In between these examples, there exist several outcomes that need further investigation of *e.g.* biological availability of heavy metals and microbial community analysis, to better predict the risk to soil microbial functions.

Conclusions

- Anthropogenically introduced heavy metals continued to have negative effects on the environment of organic forest soil around a heavily trafficked road and a smelter plant, despite reductions in heavy metal emissions in recent years.

- Characterisation of microbial risk from heavy metals in mor layers should not be based on single monitoring techniques. Instead, integrated examination of heavy metal distribution, field dose-response relationships, laboratory-derived effect doses and microbial community structure shifts in the evaluation of microbial risk in mor layers are recommended on the basis of results presented in this thesis.

- Assessment of heavy metal distribution is the first step in studies of soil environmental health. It involves characterisation of background heavy metal concentration and solubility patterns in soil.

- Field dose-response relationships provide first-hand information on field environmental status. However, evaluation of metal effects in dose-response relationships in the field is confounded by covariation among metals.

- Laboratory studies provide only single effects from heavy metals that are not easily comparable with the situation in the field.

- Shifts in microbial community is an important aspect in soil microbial risk assessment.

- Hg mobilisation from the mor layers is closely associated with DOC mobilisation due to the strong positive correlation between dissolved Hg and DOC. The ratio of Hg content in organic matter in solution relative to that in solids was found to remain constant between forest sites, despite differences in Hg deposition.

Svensk sammanfattning

Föroreningar av tungmetaller i det övre organiska markskiktet (det så kallade mårskiktet) i skogen undersöks i denna avhandling. Tungmetaller som sprids i naturen utgör en allvarlig miljörisk som har fortgått under mycket lång tid. Effekterna av förhöjda koncentrationer av tungmetaller är nu tydliga inom flera olika ekosystem.

Skogsmarken anses som extra känslig för luftspridda tungmetaller. Barrskogen fungerar som ett effektivt filter som fångar upp partiklar och andra luftburna ämnen i luften på barren som sedan transporteras vidare till marken. Markens känslighet beror dels på det mikrobiella samhällets stora känslighet för tungmetallstörningar, dels på markens förmåga att fasthålla tungmetallerna i det organiska materialet. Denna avhandling diskuterar hur man kan göra en bedömning av miljön i mårskikt som är förorenade av tungmetaller. Avhandlingen innehåller fyra separata studier. De två första undersökningarna visar på hur tungmetaller som sprids inom ett begränsat område påverkar markbiologin. Den första av dessa undersökningar är gjord i närheten av en motorväg i Stockholm och den andra är gjord vid ett stort järnsmältverk i Avesta. Ytterligare två undersökningar gjordes på markmaterial i områden med litet nedfall av tungmetaller. I den ena av dessa undersökningarna tillfördes tungmetaller experimentellt till markprover i laboratoriet för att undersöka hur koncentrationer av tungmetaller påverkar markbiologin. I den andra studien undersöktes hur kvicksilver transporteras tillsammans med löst organiskt kol från mårskiktet.

Vid motorvägen kunde man finna tydliga förhöjningar av framförallt bly och koppar. Vid smältverket var molybden, krom och nickel de tungmetaller som uppvisade de största förhöjningarna. Nära smältverket var markkemin också starkt påverkad av alkaliskt nedfall som ökade pH till onormalt höga nivåer. Denna störning i markkemin förändrade exponeringen av den lösta tungmetallfraktionen avsevärt. Molybden var mycket lösligt vid det höga pH som fanns i den alkalint störda marken, medan krom och nickel endast var lite lösliga. Vid motorvägen syntes ingen skillnad i löslighet mellan de ytor som undersöktes.

Fältundersökningar av samband mellan metallkoncentrationer och markrespiration inom små ytor (50x50 m) användes för att påvisa möjliga negativa effekter av tungmetallerna på markrespiration. Inom ytor nära utsläppskällorna kunde inga negativa samband påvisas (sådana samband skulle kunna indikera en tungmetalleffekt på markens mikrobiella aktivitet). Negativa samband mellan metallkoncentrationer och markrespiration kunde först upptäckas inom ytor längre ifrån utsläppskällorna.

För att öka möjligheterna att utföra en god miljöbedömning av skogsmarken bestämdes och användes effektnivåer från laboratorieförsök. Utöver markens mikrobiella aktivitet undersöktes också det mikrobiella samhället genom att analysera sammansättningen av långa fettsyror i markmikrobernas cellväggar (PLFA). Denna metod är ett effektivt sätt att utveckla den biologiska

riskbedömningen, eftersom förändringar i mikrobiell aktivitet inte alltid syns när marken utsätts för metallföroreningar. En förändring i den mikrobiella sammansättningen skulle däremot kunna påvisa en tungmetalleffekt. Resultaten från denna undersökning visar att förändringar i den mikrobiella aktiviteten var starkt korrelerad med förändringar i det mikrobiella samhällets struktur, samt att förändringarna i sammansättningen av det mikrobiella samhället var i stort sett lika för alla metaller vid metalltillsats.

Det regionala mönstret av deposition av kvicksilver visar en tydlig syd-nord gradient där södra Sverige uppvisar en hög deposition av kvicksilver som sedan minskar norrut. Denna gradient avspeglas också i marken där samma tydliga gradient syns i koncentrationen av kvicksilver i mårskiktet. Kvicksilver anses vara en extra giftig tungmetall för markens mikrobiella aktivitet. Negativa effekter på markrespirationen kan påvisas vid små förhöjningar över den naturliga bakgrundsnivån. Genom internationella överenskommelser har utsläppen och depositionen av kvicksilver minskat. Samma positiva trend har dock inte kunnat påvisas för koncentrationer av kvicksilver i måskiktet. För att bedöma hur skogsmarken återhämtar sig när nedfallet av kvicksilver minskar utfördes mätningar av kvicksilver och kol i det fasta mårmaterialet samt kontinuerliga mätningar på koncentrationer av kvicksilver, samt löst organiskt kol, i markvatten på en lokal i södra Sverige samt en lokal i norra Sverige. Resultaten från denna undersökning visade att kvicksilver som är bundet till det fasta organiska materialet har samma halt som i markvattnets organiska materialet. Fördelningen av kvicksilver i fast respektive löst organiskt material var alltså konstant i de undersökta områdena oavsett skillnaderna i deposition. Detta förhållande kommer att förbättra framtida modeller som beräknar hur skogsmarken återhämtar sig från den minskande kvicksilver depositionen.

Denna avhandling föreslår en modell för att bedöma risk för hur mårens mikrobiologi påverkas av tungmetaller. Fältundersökningar kan visa om markens mikrobiologi påverkas av föroreningar men samvariation mellan tungmetaller gör det svårt att utvärdera vilken tungmetall som utgör den potentiella risken för markens mikrobiologi. För att förbättra utvärderingen av hur enskilda tungmetaller påverkar markrespiration jämförs experimentella effektkoncentrationer med uppmätta koncentrationer i fält. Genom att samordna resultat från laboratorieundersökningar med fältundersökningar går det att göra en bedömning av skogsmarkens miljötillstånd.

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